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Integrative species delimitation of the widespread North American jumping mice (Zapodinae)

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ABSTRACT

Delimiting species can be challenging, but is a key step for the critical examination of evolutionary history and for prioritizing conservation efforts. Because systematic relationships are often determined iteratively using tests based on taxonomy, such methods can fail to detect cryptic variation and result in biased conclusions. Conversely, discovery-based approaches provide a powerful way to define operational taxonomic units and test species boundaries. We compare both approaches (taxonomy-based delimitation – TBD and discovery-based delimitation – DBD) within North American jumping mice (Zapodinae) using broad sampling, multilocus analyses, and ecological tests. This group diversified through the dynamic glacial-interglacial periods of the Quaternary and phylogeographic tests reveal 28 lineages that correspond poorly with current taxonomy (4 species, 32 nominal subspecies). However, neither the 4-species or 28-lineage hypotheses are optimal for species-level classification. Rather, information theoretic approaches (Bayes Factors) indicate a 15-species hypothesis is best for characterizing genetic variation in this group, with subsequent iterative pairwise ecological tests failing to confirm four species pairs. Taken together, evolutionary and ecological tests capture divergence among 11 putative species that, if upheld by additional tests, will lead to taxonomic revision and reevaluation of conservation plans.

1. Introduction

Understanding how divergence ultimately leads to speciation is a central question in evolutionary biology. Application of molecular phylogenetic approaches routinely reveals patterns of differentiation that improve our collective understanding of both the origin and timing of divergence, and when coupled with independent data (*e.g.*, ecological variation) can expose underlying diversification processes (Crandall et al., 2000; Wiley and Lieberman, 2011). Refining our understanding of evolutionary relationships and the ecogeographic limits of taxa provides a requisite context for other fields (Bernardo, 2011; Riddle and Hafner, 1999), particularly conservation (Haig et al., 2006). Species delimitation approaches aim to refine our understanding of diversification processes (Camargo and Sites, 2013; Carstens et al., 2013; Jackson et al., 2016). Nevertheless, because most species likely evolve in allopatry, a complication for any delimitation technique is whether sufficient evolutionary and ecological changes have accrued to allow species detection (Coyne and Orr, 2004; de Queiroz, 2007; Mayr, 1963). For example, species long isolated by a barrier can accumulate neutrally evolving genetic differences, but because environments may remain similar, few ecological or even morphological differences may be apparent. Conversely, recently diverged species can rapidly adapt to distinctive localized conditions, often resulting in ecological and morphological divergence, but few neutrally evolving genetic changes. Because of these different evolutionary signatures, delimitations based solely on a single class of characters may fail to detect speciation. Therefore, species delimitation techniques that integrate across multiple datasets should be favored (Carstens et al., 2013; Sukumaran and Knowles, 2017).

Traditionally taxonomists have faced a choice among a variety of competing conceptual approaches to either delimit or describe species (Camargo and Sites, 2013; Sites and Marshall, 2004; Wiens, 2007). Species delimitation has experienced resurgence due to new integrative approaches (Carstens et al., 2013; Edwards and Knowles, 2014; Hope et al., 2016) that are based on hypothesis tests that better capture spatiotemporal signatures of

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evolutionary history (Grummer et al., 2013; Padial et al., 2010; Yang and Rannala, 2010). Analyses that integrate diverse datasets and apply explicit tests are expected to be most powerful when coupled with coalescence methods (Carstens and Dewey, 2010; Fujita et al., 2012). There are several species delimitation approaches, therefore the choice of approach for characterizing operational taxonomic units (OTUs) for testing for the signal of speciation is nontrivial (Carstens et al., 2013; Sukumaran and Knowles, 2017). Approaches to species delimitation include two broad techniques. Unguided approaches attempt to jointly estimate OTUs and test phylogenetic relationships, but they remain problematic and have received less attention despite active development (Ence and Carstens, 2011; Jones et al., 2015; O'Meara, 2009; Yang and Rannala, 2014). Conversely, guided approaches require OTUs from the outset and are now common (Edwards and Knowles, 2014; Toprak et al., 2016). Unguided approaches will likely supplant guided approaches, but there currently remains no consensus over an optimal technique. Critically, guided approaches that depend on existing taxonomy from the outset require careful consideration, because a flawed taxonomic hypothesis can introduce undesirable bias (Olave et al., 2014).

Guided approaches use two broadly defined methods to establish OTUs (Ence and Carstens, 2011). The first uses taxonomy usually drawn from an existing premise of morphologically identified species and subspecies. Taxonomy-based delimitation (TBD), also called validation-based approaches (O'Meara, 2009), assesses taxonomic units by applying validation tests of described variation. The second method uses discovery-based delimitation (DBD), also called substructure detection methods (Rannala, 2015). When applying DBD, a set of OTUs or 'candidate species' is identified using genetic clustering or assignment tests, thereby providing a starting point to compare and evaluate alternative hypotheses of systematic relationships (Camargo and Sites, 2013; Edwards and Knowles, 2014; Leache and Fujita, 2010). In some cases, taxonomic and discovery-based approaches produce similar results, but in situations where taxonomy is suspect, DBD may provide alternative perspectives on species limits. Both TBD and DBD require robust tests using multiple datasets rather than relying on a singular dataset (Fujita et al., 2012; Rannala, 2015).

Morphology-based taxonomy often forms *a priori* hypotheses in species delimitations in vertebrates. Mammalian systems have been exemplar models for species-tree approaches and existing taxonomy frequently guides sampling (Ence and Carstens, 2010; Heled and Drummond, 2010). While taxonomy-based tests may represent a natural progression from earlier studies (*i.e.*, validation of described morphological variation), they may also violate assumptions of some analyses, especially when paraphyly or gene flow are present. Furthermore, tests predicated on previously described variation often miss cryptic variation (Leache et al., 2009; Pyron and Burbrink, 2009). Tests predicated solely on morphology may best reflect phenotypic responses to environments, but may also fail to provide the optimal basis for exploring evolutionary history or establishing taxonomy (Gotthard and Nylin, 1995; Mayr, 1956; Simpson, 1951), although there are exceptions (Hoekstra et al., 2005; Patton and Smith, 1994). Consequently, delimitation approaches that rely exclusively on described phenotypic variation (*i.e.*, TBD) should be validated with independent data and analyses (Carstens et al., 2013; Fujita et al., 2012; Rannala, 2015).

In this study, we unite signatures of spatial, genetic, and ecological divergence to compare and evaluate alternative hypotheses of species limits in a continentally-distributed clade of rodents. More specifically, we apply guided approaches to species-delimitation and evaluate whether taxonomy optimally reflects evolutionary history. We generate hypotheses that originate from phylogeographic signatures (DBD) that we compare to morphologically-based taxonomy (TBD). After contrasting DBD and TBD approaches using genetic data, we then apply iterative ecological tests to assess genetic conclusions. Taken together, we present a new taxonomic hypothesis that better reflects phylogeny, biogeography, ecology, and has immediate implications for conservation.

1.1. Study system

Jumping mice (Dipodidae, Zapodinae) are broadly distributed (Fig. 1) from northern Alaska and Canada, south to the montane areas of the American Southwest and Appalachians (Hall, 1981; Krutzsch, 1954). Zapodines include two genera, four species, and 32 subspecies that are spread over 30 North American ecoregions (SF1, SF2; (Holden and Musser, 2005; Krutzsch, 1954). Such a diverse set of environments provides an excellent system to assess how physical barriers, geographic isolation, and environmental variability interact to influence divergence (Pigot et al., 2010).

Phylogeographic studies have uncovered cryptic variation and paraphyly between the western jumping mouse (*Zapus princeps*) and Pacific jumping mouse (*Z. trinotatus* (Malaney et al., 2013). Multiple Pacific coastal forest and montane-associated lineages have significantly deeper divergences than observed within the more broadly distributed grassland-associated meadow jumping mouse (*Z. hudsonius*; (King et al., 2006; Malaney and Cook, 2013; Malaney et al., 2012; Ramey et al., 2005). Similarly, the woodland jumping mouse (*Napaeozapus insignis*) reflects a deep allopatric divergence associated with the temperate forests of eastern North America (Malaney and Cook, 2013). When combined, at least four, major phylogeographic patterns exist across the wide distribution of jumping mice (Malaney and Cook, 2013) that are generally shared with several other widespread mammals, suggesting a common response to climate fluctuations in northern-temperate mammals (Malaney, 2012). A consensus among jumping mice phylogeographic studies is that there is deep and often cryptic variation within species, and evidence that evolutionary lineages are discordant with taxonomy (Himes and Kenagy, 2013; King et al., 2006; Malaney et al., 2013; Malaney and Cook, 2013; Malaney et al., 2012; Ramey et al., 2005). Consequently, testing taxonomic hypotheses against phylogeographic hypotheses is crucial for optimally characterizing variation and defining conservation priorities. More specifically, this clade is ideal for testing DBD and TBD approaches because these phylogeographic signals (Himes and Kenagy, 2013; Malaney et al., 2013; Malaney and Cook, 2013) challenge existing classifications (Hall, 1981; Holden and Musser, 2005; Krutzsch, 1954).

2. Methods

2.1. Generalized approach

We used molecular sequences and ecological variables to establish and test candidate species (discovery-based delimitation, DBD) and to test described variation (taxonomy-based delimitation, TBD), using broad geographic sampling that includes all recognized species and subspecies. For the discovery-based hypotheses, we apply four approaches expected to capture the spectrum of evolutionary and ecological variation and that reasonably sample patterns of speciation (Sukumaran and Knowles, 2017). We then evaluate hypotheses generated from both TBD and DBD using multilocus species-trees and Bayes Factor tests to identify the hypothesis that best reflects evolutionarily significant diversity (Grummer et al., 2013). Finally, we iteratively apply pairwise ecological tests as an approximation of niche conservatism/divergence among candidate species (sister taxa) predicted by the optimal hypothesis.